

FOOD AND FARMING GROUP

Veterinary Sciences Team

EPIDEMIOLOGY REPORT

Highly Pathogenic Avian Influenza H7N7, Oxfordshire, June 2008
Situation at 12.30pm Wednesday 2nd July



Prepared by the National Emergency Epidemiology Group (NEEG)

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Executive Summary

1. Following a report of suspected avian notifiable disease in laying hens in Oxfordshire on 2 June 2008, highly pathogenic (HP) H7N7 avian influenza (AI) infection was confirmed on 4 June 2008. At the time of writing (2 July), twenty further reported cases from across GB have been negated either on clinical grounds or after laboratory examination of samples.
2. This report presents the results of additional epidemiological investigations and analyses carried out since the first epidemiology report for this outbreak, published on 17 June 2008, and available at <http://www.defra.gov.uk/animalh/diseases/notifiable/disease/ai/latest-situation/index.htm>.
3. On 2 July, it appears that the infection is confined to a single premises. Infection was ruled out at both premises of origin of the affected birds. Investigations have found no evidence of infection on any contact (tracings) or geographically close premises, and there has been no evidence of spread of infection to any other premises to date.
4. Clinical evidence from the farm's records supports virological data that the HPAI infection derived from a pre-existing Low Pathogenicity AI (LPAI) H7 virus present on the premises. Laboratory investigations provide support for this hypothesis. H7 viral RNA was detected from faecal samples collected from beneath Sheds 1 and 2. Further molecular analysis yielded, from one of these samples, an identical haemagglutinin (HA) cleavage site sequence to that of an unrelated H7 influenza virus from 1976 from Australia, which had been shown by *in vivo* testing to be a LPAI virus.
5. The two most likely hypotheses investigated for the source of the outbreak were:
 - i. Unidentified AI in domestic poultry premises in Great Britain, associated either by proximity or potential contact, or
 - ii. AI in wildlife in contact with the IP.

All investigations have been completed and neither of these two routes could be categorically ruled out as the source of infection.

6. Wild bird activity in general was described as being low around the IP by expert ornithologists. Overall it can be concluded that wild bird species found on the IP did not pose a high risk for introduction of AI.
7. It should be noted that a population of mallard ducks introduced onto a pond on the premises in 2007 were seen to be mixing with the poultry. Laboratory testing of samples collected from a small proportion of these ducks was negative, but significant uncertainty remains as to the true AI status of the mallard population due to i) the limited availability of suitable samples from the ducks, and ii) the inevitable time that passed before sampling the mallards following the onset of clinical signs in the laying hens. The latter would have significantly reduced the likelihood of detecting avian influenza virus.
8. The detection of an HA cleavage site amino acid sequence identical to an LPAI virus that was previously isolated from ducks, indicates that the mallard ducks on the IP are a plausible source of infection. Furthermore, this previously isolated virus was believed to be the LPAI progenitor strain for an HPAI outbreak in poultry in Australia in 1976.
9. If the source of infection was another domestic poultry flock, then, with all the investigations completed, it remains unidentified. Despite intense field investigations and sampling, a number of scenarios might explain the absence of positive results: i) unidentified contacts, ii) lower than detectable level of LPAI infection on premises within the PZ and/or identified contacts and iii) birds having been sent to slaughter or moved off prior to investigations commencing.

Introduction

10. This report updates the initial epidemiology report, published on 17 June 2008, and completes reporting of all the epidemiological investigations carried out, which seek to explain the outbreak of highly pathogenic avian influenza (HPAI) H7N7 infection in free-range laying hens on a premises near Banbury, Oxfordshire. The characteristics and events on the farm itself described in the early report are not repeated in this report, which focuses on those areas of the investigation for which new evidence is available.

Description of the outbreak

The Infected Premises (IP)

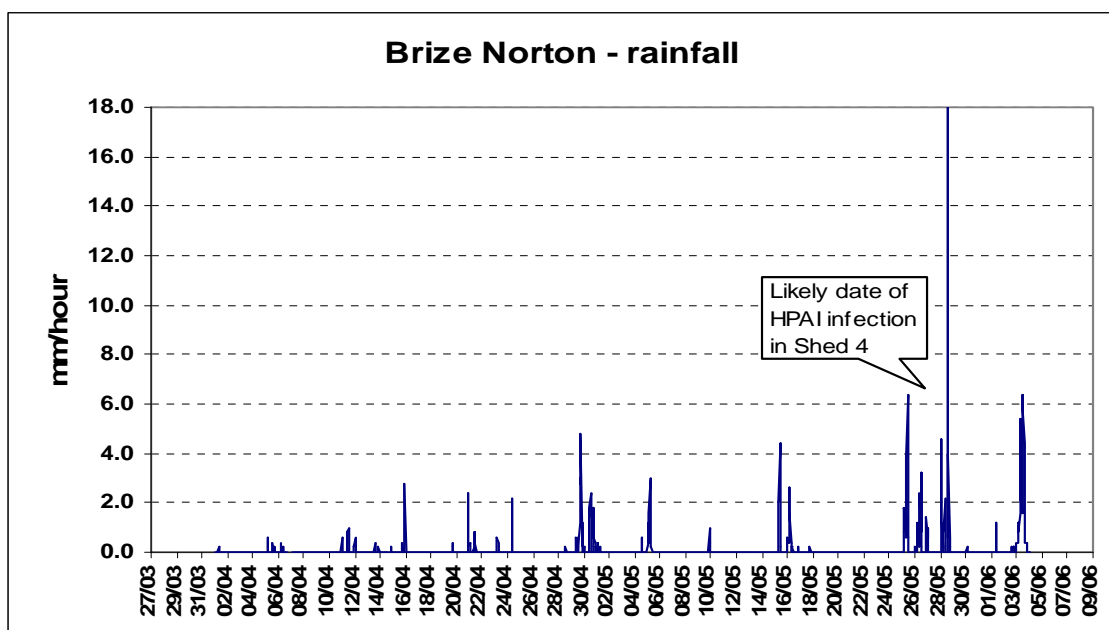
A description of the IP, structured according to the epidemiological triad (animal, time and space), was presented in the initial epidemiology report.

11. A further ornithological inspection of the IP and its surroundings reported small numbers of expected lowland farmland species. These were considered to almost certainly all be breeding birds, with very restricted home ranges at this time of year, and would only be expected to roam hundreds of metres at most. All bar

one of the species observed are predominantly resident in Britain, with Common Whitethroat being a long distance migrant, wintering in sub-Saharan Africa.

12. The IP is surrounded by drilled fields. The most recently drilled fields to the west of the IP held single figures of Rook and Carrion Crow. There were no concentrations of corvids on any of the drilled fields, though this may be because birds were concentrated at a landfill site (see below). Apparently, during the ploughing of the now newly drilled fields in May, farm workers reported large numbers of gulls following the plough. No gulls have been seen in the area since. A landfill site nearby the IP attracted corvids, but not gulls. There were no active rookeries around the IP or the landfill site. The landfill site has been accepting domestic waste, and this has greatly increased the number of corvids using the site. From the road only, at least 1,560 corvids could be seen, approximately 60:40 Rook:Jackdaw.
13. The five pigs on the IP were clinically inspected and sampled. Laboratory testing confirmed both the absence of type A influenza virus infection and the absence of exposure to infection.
14. Three dogs were also kept on the IP. According to the IP's owner the dogs were healthy and have been for the past months. No other pets were kept on the premises.
15. A meteorological assessment was conducted to investigate the weather conditions during the period 01/04/2008 to 03/06/2008. Meteorological data was obtained from the Royal Air Force (RAF) Brize Norton station located approximately 28 km to the south and west of the IP. The most significant feature of the last two weeks of May was the heavy rainfall which occurred between 25 and 28 May (Figure 1). Rainfall was recorded on 66 hours out of a maximum of 96 hours. On these four days alone around 130% of an average May rainfall was recorded. The precipitation on 30 April was considered relatively small.

Figure 1: Rainfall recorded at closest weather station (RAF Brize Norton).



16. The high rainfall between 25 and 28 May could have been a contributing factor for the speed of spread of infection and was also considered in the source investigations. The first half of May, when LPAI is believed to have been introduced, shows no significant meteorological features.

Control Measures

17. Suspicion of avian notifiable disease was reported on 2 June 2008, and immediate restrictions, to reduce the risk of spread of disease, were placed on the suspected premises. On 3 June 2008 a Temporary Control Zone (TCZ) with an inner zone with a minimum radius of 3km, and an outer zone with a minimum radius of 10 km, from the premises, was established around the IP after preliminary tests were positive for H7 AI. This temporary measure was replaced by a protection zone (PZ) and a surveillance zone (SZ), respecting the same boundaries as the temporary measures, on 4 June 2008, after HPAI H7N7 was confirmed. On 28 June, after confirmation that all investigations into the possibility of the presence of AI within the PZ were negative, PZ restrictions were lifted and holdings in this area became subject to the SZ restrictions.

18. Preliminary disinfection of the IP was completed on 7 June. Secondary cleansing and disinfection is in progress at the IP.

19. All premises within the PZ and known to have poultry were:

- Clinically inspected by an Animal Health veterinary officer who also checked production and medicines records in order to identify any possible manifestation of infection with AI virus, and
- Advised owners to remain vigilant and to report any possible clinical manifestation in their birds which might be suggestive of avian notifiable disease.
- Commercial premises were re-visited every 5 days during which time they remained under the restrictions extant on declaration of the PZ¹.
- If ducks or geese were present, or if the records were poor, inadequate or not available, such that the VO could not fully assess the clinical history then sampling for laboratory investigation for AI was carried out in accordance with instructions (see Appendix 1).

20. 63 premises were identified within the PZ, five of which did not keep any susceptible species. Three are commercial premises and the remaining 55 are non-commercial. The number of animals in the 55 non-commercial premises ranges between 1 and 36. All 58 premises were visited and assessed by field staff. Veterinary Officers carried out a comprehensive assessment of production and medicine records (where available) looking for any possible manifestation of AI (e.g. increase in mortality, reduced egg production or change in medicine usage).

¹ Avian Influenza and Influenza of Avian Origin in Mammals (England) (No.2) Order 2006; Article 28(1).

21. Visits by officials to poultry premises within the PZ started on 8 June. Table 1 shows the number of poultry premises within the PZ and SZ by species and type of production. All the premises in the PZ have been visited and no evidence of infection has been found.

Table 1. Description of the susceptible domestic bird population in the PZ and SZ

<i>Type of premises</i>	<i>PZ</i>		<i>SZ</i>	
	<i>No. of Premises</i>	<i>No. of Birds</i>	<i>No. of Premises</i>	<i>No. of Birds</i>
Outdoor premises	22	185	28	20898
Indoor premises	30	333	14	33387
Unknown housing premises	6	2744	28	unknown
No Stock	5		1	
TOTAL	63	3262	71	>54285
Of which				
Ducks and geese	14	541	15	1152
Game (inc pheasants, quail, guinea fowl)	4	2163	16	12229
Total for premises with stock	58	3262	70	>54285

22. Samples were collected from birds on all three commercial premises for laboratory testing to rule out the presence of AI. Based on the veterinary risk assessments conducted by field staff, of the 55 non-commercial premises, birds on twelve were sampled and tested to rule out the presence of AI infection. These assessments considered both the probability of exposure to wild waterfowl and the probability that inspection could fail to detect AI. Three main factors were considered when assessing the need for laboratory testing: i) the proximity of an open source of water within 500 metres of the premises with reported wild waterfowl, ii) whether the premises kept ducks and/or geese, and iii) if records were poor, inadequate or not available at the time of the field staff visits.

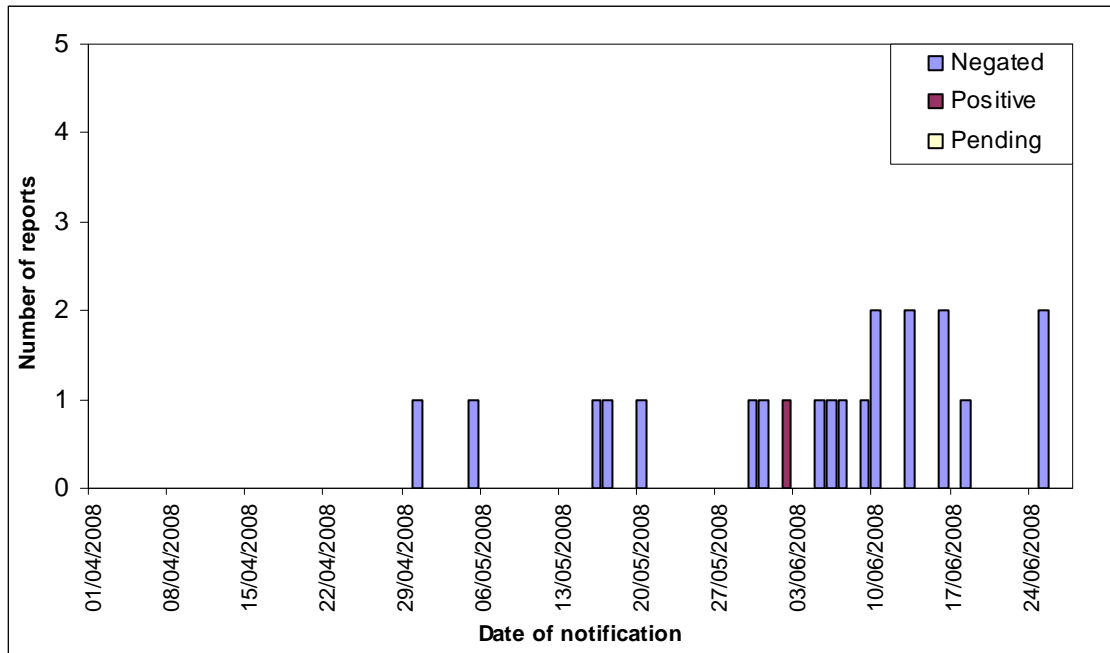
23. Assessments and sampling were carried out according to the instructions described in Appendix 1. Test results from all fifteen premises sampled within the PZ were negative for AI. Samples were taken after 21 June 2008 (14 days after completion of preliminary disinfection on the IP) to enable detection of seroconversion if present.

24. Within the Surveillance Zone (SZ):

- A list of 71 commercial poultry premises was compiled from records in the GB Poultry Register, VETNET and held by Local authorities (Table 1).
- A letter was sent to the 71 premises identified above to i) remind them of the restrictions that apply in the SZ, ii) advise them to remain vigilant for evidence of AI and iii) explain the timeline for potential changes in the restriction zones.

25. Thirteen suspect cases of avian notifiable disease (AND) were reported between confirmation of the IP and 25 June across GB (Figure 1). Three of these cases were reported within the PZ/SZ. All reported cases were negated either on clinical grounds or after completion of laboratory testing.

Figure 1: Number & status of holdings reporting suspicion of AND across GB, 1 April 2008 to 25 June 2008



Investigations

Within the IP

26. Laboratory results from samples collected at the initial report on 2 June 2008, and from those collected at the time of statutory culling on 4-5 June 2008 are shown in Appendix 2. Analysis of the data suggests that at the time of the initial report (2 June 2008) there was serological and clinical evidence that infection with a presumed LPAI H7 virus had been present on the IP for at least 12 days (Shed 1) prior to sampling. Active H7 infection (evidence of the presence of the virus) was also detected from samples collected from birds in Sheds 3 and 4. At culling, random sampling of a larger number of birds in all four sheds confirmed widespread seroconversion (using virus from the IP as the antigen for HI testing) in Sheds 1 and 3, exposure to the putative LPAI progenitor virus in Shed 2 and the presence of active H7 infection with HPAI virus in all four sheds. The mortality rate was highest in Shed 3 although more birds died in Shed 4 due to the greater population size.

27. Sera collected at the time of initial veterinary inquiry (2 June 2008) and at culling (4-5 June 2008) has revealed different proportions of seropositive birds to H7 virus in each of the four sheds on the IP. It is noteworthy that at the initial report birds that

were clinically ill were included in those selected for testing, and may therefore have been sampled too early in the course of disease for there to be a detectable antibody response (as clinical signs precede seroconversion). At culling, field reports confirm a more random selection of birds for sampling, suggesting that the recorded seroprevalence in each shed is more likely to reflect the actual proportion of birds that had detectable levels of antibodies, indicative of previous exposure to H7 virus infection. The very low proportion of seropositive birds and the high mortality rate in Shed 4 is consistent with HPAI H7N7 infection of a fully susceptible poultry population, resulting in an acute and rapid escalation in mortality.

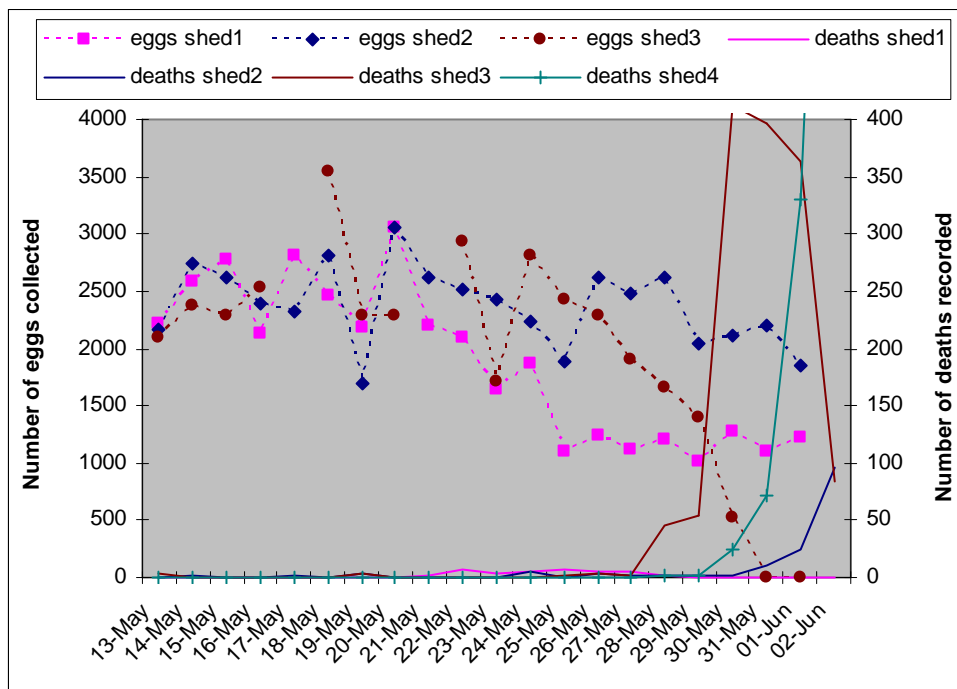
28. The combined analysis of laboratory results and descriptive epidemiological evidence supports the hypothesis that LPAI H7 virus infection was introduced into the susceptible free-range layer population in Shed 1 on or around the second week of May 2008. This resulted in the development of clinical disease in Shed 1, first evident on 21 May 2008 (Figure 2), and lasting for approximately two weeks.
29. Serological evidence, supported by clinical records, further substantiates the spread of LPAI H7 infection to Shed 2 and to Shed 3. The rapid escalation in the severity of clinical disease in Shed 3, notably the daily mortality from 29 May 2008 (Figure 2), is consistent with the occurrence of at least one mutation of the presumed LPAI H7 virus to high pathogenicity in the presence of a partially immune poultry population in Shed 3. The events leading to the eventual acquisition of genotypes and phenotypes of high pathogenicity occurred 7-10 days after the initial clinical presentation in Shed 1. Furthermore, following the acquisition of the HPAI genotype in Shed 3, approximately half of the birds died over a 5-day period, which suggests that this proportion of the poultry population in Shed 3 were naïve and had not seroconverted to prior LPAI H7 infection at that time.
30. Immediate spread (on or around 29 May) of the HPAI H7N7 virus then occurred from Shed 3 into a fully susceptible population in Shed 4 resulting in an acute and severe clinical deterioration (putative HPAI virus incubation period of 48 hours). Genetic analysis of viruses suggests the onward spread of the HPAI virus back into Shed 1 and Shed 2 at this time.
31. To identify the progenitor LPAI H7 virus presumed to have infected the flock, ten faecal samples were collected after depopulation from beneath the slatted areas of Sheds 1 and 2. H7 viral RNA was detected by real-time RT-PCR (RRT-PCR) from one of the samples. Further molecular analysis yielded an HA cleavage site nucleotide sequence of PEIPKKRGLF, which has only been reported previously in an influenza virus isolated in Australia - A/duck/Victoria/76 (although the geographical origin of this virus is immaterial to the current outbreak and its analysis).
32. Westbury and others (1979)² demonstrated the avirulent nature of this virus which, when inoculated by the intra-nasal route caused no disease in non-SPF 14-week-old chickens, 18-week-old turkeys and 2-year-old ducks. Furthermore, the A/duck/Victoria/76 virus was considered to be the progenitor LPAI strain for the HPAI outbreak affecting poultry in Victoria, Australia in 1976. The pathotype of this virus has also been previously determined by intravenous pathogenicity index (IVPI)

² Westbury HA, Turner AJ, Kovesdy L (1979). The pathogenicity of three Australian fowl plague viruses for chickens, turkeys and ducks. *Veterinary Microbiology* 4, 223-234.

testing in SPF chickens, which clearly demonstrated the virus to be of low pathogenicity, with an IVPI of 0.0 (Paul Selleck, personal communication).

33. In combination with the descriptive and clinical evidence, it is reasonable to conclude that an LPAI H7 virus was present on the IP, based on demonstration of H7 viral RNA with an HA cleavage site sequence identical to a virus previously shown by *in vivo* testing to be an LPAI virus.
34. In addition, there is no evidence to support the hypothesis of a sole introduction of an HPAI H7 virus to the IP in the absence of a LPAI H7 progenitor strain since the pathogenic and genetic characteristics of the virus are not consistent with fully susceptible birds surviving natural infection to allow for the induction of specific H7 antibodies. Yet there was widespread seroconversion among surviving birds in three of the four sheds due to previous exposure to the putative LPAI H7 virus.

Figure 2: Mortality and egg-production in each shed (except Shed 4, only mortality shown)



Source investigations

35. Epidemiological investigations on the IP focused on the identification of possible sources of infection derived from the known epidemiology of AI, and potential risk factors unique to this farm. The hypotheses for source that were identified were:
- i. AI in wildlife in contact with the IP.
 - ii. AI in associated premises in Great Britain, either by proximity or other contacts.
 - iii. Introduction by purchased birds from the source farms.
 - iv. Unidentified infection in product at local slaughterhouse or similar premises, moved to the IP by wildlife.

- v. Infected imports from other countries of live poultry, hatching eggs or poultry products into the locality around the IP, or otherwise epidemiologically associated with the IP.
- vi. Escape or reversion of virus used for vaccine, as inactivated H7N7 vaccine can be licensed for use in zoo birds.
- vii. Escape from laboratories licensed to use live virus for research and/or diagnosis.

Hypothesis (i): Wild bird or/and wildlife source

36. The clinical evidence, supported by laboratory results, suggested the entrance of putative H7 LPAI infection initially into Shed 1. This shed is distant from the main egg management activity in Shed 4, and from the vehicular access to the site. It may also be more exposed to wild animals and birds than the other sheds. Expert ornithological inspection reported that upslope to the east of Shed 1 is a thick hedge which had a denser understory than most other hedges, and hence may be more suitable as nesting habitat for small passerines. The density of birds in the hedge was still low though, and no greater than in other hedgerows around the IP. Feathers found around Shed 1 were identified as Woodpigeon and corvid, both of which were present in the area. The only evidence of presence of birds directly around Shed 1 was a single corvid feather inside one of the scratching areas, though it was presumed that this had been carried in by wind or man. There was limited access for small passerines into Shed 1 along the end of the roof apex, but there was no evidence of wild bird presence in the Shed itself. It was concluded that there was no evidence to suggest that there is a significant difference in wild bird contacts between poultry in Shed 1 and on the rest of the IP.
37. The presence of gulls in early May, reported by farm workers, and that of corvids on the IP and surrounding land was considered by ornithological experts to be a very low risk.
38. The presence of an open source of water in the IP was assessed in the context of how frequent this feature was in the surrounding premises within the PZ and among those identified as tracings. Field staff collected information on whether there was an open source of water within 500 metres of all premises visited during the investigations for source and spread. Table 2 shows that 61% of premises in the PZ with susceptible species where this enquiry was made had open water within 500m of the domestic birds. This shows that the open water on the IP was not unusual among premises in this area. Based on these results, the pond near Shed 1 in the IP was not considered an epidemiologically significant feature of the IP. This does not dismiss the potential importance of the network of ponds around the IP in relation to the overall spread of the virus by a wild bird source.

Table 2: Distribution of the presence of bodies of water within 500 metres of susceptible stock on premises within the PZ and identified as tracings

	Body of water Present (%)	Body of water Absent (%)	Total Premises
PZ	34 (61)	22 (39)	56
Tracings	15 (45)	18 (55)	33

39. At the time of the outbreak, 30 mallard ducks, out of an initial group of 100 birds reared for sport shooting introduced onto a pond near one of the mobile sheds (Shed 1) in 2007, were present on the IP. These ducks were seen to be mixing with the poultry. The possibility of these resident ducks mixing with other birds including waterfowl at other nearby locations and bodies of water (abundant in the PZ (Table 2)), cannot be ruled out. If so, this would have posed a potential risk to the poultry.
40. H7 RRT-PCR testing of cloacal and oropharyngeal swabs from the ducks (n=17) were negative confirming the absence of detectable shedding of H7 at the time of sampling (more than two weeks after the reported onset of clinical signs in the poultry in Shed 1). Serum samples (n=3) were also collected for antibody (HI) testing with no evidence of antibodies to H7 detected. It is important to highlight that, due to the low number of samples, it is not possible to draw a definitive conclusion regarding the infection status of the mallards from these results alone.
41. However, in addition to the detection of an HA cleavage site sequence of high similarity to an LPAI H7 virus, further laboratory analysis revealed a point mutation detected from two separate isolates, one each from Shed 2 and Shed 4, which has been observed during previous outbreaks of H7 viruses in chickens and turkeys and is considered to be part of the host-adaptive process following transmission from waterfowl.
42. The potentially relevant epidemiological findings, reported above, together with the combined laboratory evidence mean that the ducks remain a plausible source of infection.

Hypothesis (ii): AI in associated premises in Great Britain

43. Unidentified infection in another domestic flock in GB in contact with the IP through:
- i. Egg-collection routes: The three premises on the egg collection route, two of which had susceptible stock, were inspected. There was no evidence of AI on them.
 - ii. Close proximity: Investigations on the adjacent premises which kept pheasants and ducks, including laboratory testing of samples, were all negative.
 - iii. Moderate proximity: All premises in the PZ with susceptible stock have been visited (see section on PZ) and no evidence of AI had been found.
 - iv. Lorry route for feed deliveries: Feed deliveries that took place in the relevant time window and tracings were investigated. No evidence of AI has been found on premises exposed to this risk.
 - v. Premises identified as a result of people in contact with poultry entering into the IP within the tracing window: These premises were included in the tracing prioritisation and tracing visits were conducted. No evidence of AI has been found on premises exposed to this risk.
44. Despite intense field investigations and sampling, there was no evidence indicating the presence of AI in any of the potentially exposed premises. Although

HPAI, due to its extreme clinical effects, would not go undetected for this length of time in a susceptible population, the possibility that LPAI was present on another domestic premises cannot be completely ruled out. Possible scenarios include: i) epidemiologically important contacts might have not been identified, ii) the sensitivity of sampling and/or clinical inspections might have not been sufficient to detect unusually low levels of LPAI infection on investigated premises and iii) birds having been sent to slaughter or moved off prior to investigations commencing.

45. Additional evidence supporting the point above comes from the EU poultry surveillance/survey programme for AI in which the UK has participated since 2004. This serological survey is designed to detect infection with LPAI H5/H7 in different poultry sectors (e.g laying hens, turkeys, ducks, etc.) assuming 5% of flocks are infected and a within-flock prevalence of 30%. Through this survey the UK occasionally identifies antibodies against LPAI H5/H7. In 2007, antibodies to avian influenza viruses of subtypes H5 and H7 were detected in samples from 10 of 340 premises. On this basis, undisclosed LPAI infection cannot be ruled out completely as a potential source of infection.

Other Hypotheses for Source

46. Completed investigations have ruled out all the remaining hypotheses:

47. Introduction by purchased birds from the source farms. The timing of infection on the IP and the differing levels of disease in each shed make it very unlikely that the birds were infected on arrival. In addition, the two farms were visited by veterinary officials and 29 birds were sampled at each (sample calculated to detect a prevalence of 10% at the 95% level of confidence). There was no evidence of clinical disease either from clinical examination or inspection of production records. All samples were negative.

48. Unidentified infection in product at local slaughterhouse or similar premises. The closest slaughterhouse is located more than 20km away and there was no evidence of contact between those premises and the IP. Fomite transfer by wild animals has been assessed as very unlikely.

49. Imports from other countries of live poultry, hatching eggs and poultry products into the locality around the IP. No links between imports and the IP have been established.

50. Inactivated H7N7 vaccine licensed for zoo birds. There is no AI vaccine production plant in the UK, and infection from birds vaccinated with purchased vaccine is considered unlikely due to the nature of the vaccine. There are no zoo premises within the PZ and SZ, and investigations have confirmed that none of the zoos to whom a licence has been issued have a link to the IP, and all are more than 50km from the IP.

51. Escape from laboratories licensed to use live virus for research and/or diagnosis. There are no such laboratories within the PZ or SZ and investigations have not revealed any links to any such facilities. The safety alert inspection by regulatory inspectors of all facilities within Great Britain working with pathogens identified

under the Specified Animal Pathogens Order revealed no breaches of legislation in facilities working to containment level 4 requirements, and no formal enforcement action was necessary. The inspection programme provided regulatory bodies and operators of the facilities with the assurance that these high hazard facilities are well managed.

Spread Investigations

Potential routes of spread outside the IP.

52. The greatest risk of spread of AI from an infected premises would be through the movement of live infected birds. No such movements had taken place.

53. A less likely, but possible mechanism of spread is through fomite transmission to premises associated with the IP. Such potential contacts (tracings) were identified from multiple sources (e.g. disposal of manure, disposal of carcasses, feed delivery vehicles, etc.). 90 tracings enquiries have been completed. These were assessed for risk of exposure following the same protocol as described for PZ premises, and 31 of them required samples to be taken for laboratory testing. All results have been received and are negative. There are no outstanding tracing enquiries.

54. In summary there is no evidence of spread of infection outside the IP.

Acknowledgements

55. We would like to thank members of the NEEG's Ornithological Expert Panel, expert ornithologists of the British Trust for Ornithology, and John Gloster from the Met Office for their prompt participation and very useful reports that have helped clarify the epidemiology of the outbreak.

56. The National Emergency Epidemiology Group includes members from Defra's Food and Farming Group, the Veterinary Laboratories Agency and the Animal Health Agency.

National Emergency Epidemiology Group
2 July 2008

Appendix 1: Sampling strategy for potentially exposed premises

('Tracings', including premises within the PZ)

Spread investigations aim at clarifying any sort of relevant epidemiological links either:

- By proximity with the IP – this applies to all premises within 3km of the IP, i.e. within the PZ, or
- Others: subject to case by case evaluation by Veterinary Officers.

The following action path was followed:

1. Clinical inspection of susceptible stock.
2. For non-waterfowl: careful and close examination of production records (where available) over previous few months looking for low level disease. If found, follow sampling protocol below. If no evidence of disease, but there is a high risk of exposure then follow sampling protocol below.
3. If waterfowl: follow sampling protocol below.

Sampling protocol:

- The aim is to identify seropositive farms. A minimum of 14 days from exposure was estimated sufficient to allow time for detectable seroconversion.
- Sampling aims to detect 15% or greater seroprevalence. This design prevalence was estimated based on the evidence from other AI outbreaks and coherent with the EU AI Diagnostic Manual recommendation of a minimum of 20 birds sampled per production unit.
- A minimum of 20 birds to be sampled per holding and a minimum of 6 birds per epidemiological group. Where there are less, then all in the group should be sampled.
- If any of the blood samples from a holding are seropositive, cloacal and oropharyngeal swab samples collected from the flock are examined for the presence of virus.

Appendix 2: Results from the IP at initial report (2 June 2008) and culling (4-5 June 2008).

Table a). At initial report (2 June 2008)

Samples	Tested			Results		
	Blood	Birds	Environmental	Blood % positive	Birds % positive**	Environmental % positive
Domestic birds (Chickens)-SHED 1	20	0	0	75%	0%	0%
Domestic birds (Chickens)-SHED 2	19	0	0	0%	0%	0%
Domestic birds (Chickens)-SHED 3	19	20*	0	5%	90%	0%
Domestic birds (Chickens)-SHED 4	20	20*	0	0%	100%	0%

* 20 birds sampled (two swabs per bird: 1 cloacal and 1 oropharyngeal).

** Positive if detected either from cloacal or oropharyngeal swabs.

Furthermore, three carcasses were collected from Shed 3 and Shed 4. Carcase samples were bulked together and tested as four pools of tissues from all birds. For Shed 3, two of the four pools of tissues were H7 RRT-PCR positive. For Shed 4, the four pools of tissues were H7 RRT-PCR positive.

Comparison of serology results needs to account for the small differences in the antigenic properties of the virus strains used in serological testing of samples from the initial veterinary enquiry and at culling.

Table b). At culling (4-5 June 2008)

Samples	Tested			Results				
	Blood	Birds*	Environmental	Blood % positive	Birds % positive**	Environmental % positive	% dead birds attributed to AI	Live birds at culling
Domestic birds (Chickens)-SHED 1	100	93	10 (faeces)	95%	17%	66%	14%	2591
Domestic birds (Chickens)-SHED 2	100	94	10 (faeces)	42%	28%	100%	5%	2856
Domestic birds (Chickens)-SHED 3	100	88	0	97%	44%	0%	45%	1649
Domestic birds (Chickens)-SHED 4	100	94	0	100%	92%	0%	15%	13571

* 100 birds sampled (two swabs per bird: 1 cloacal and 1 oropharyngeal).

** Positive if detected either from cloacal or oropharyngeal swabs.

'Live birds at culling' is the number of live birds per shed at the time of slaughter. Note that this constitutes the remaining population per shed from which birds were selected for sampling.

Furthermore, five carcasses were collected from Shed 1 and Shed 2. Carcase samples were bulked together and tested as four pools of tissues from all birds. The four pools of tissues were H7 RRT-PCR positive in both sheds.

Table c). At culling (5-7 June 2008). Waterfowl and environmental sampling.

Samples	Tested				Results			
	Blood	Swabs	Carcasses	Environmental	Blood % positive	Swabs % positive	Carcasses % positive	Environmental % positive
Waterfowl (sampled 5th,6th and 7th June)	3 (6 samples insufficient or untestable)	0	18	see below	0	N/A	0	see below
Faeces (sampled from wild waterfowl on 5th June)	N/A	N/A	N/A	6	N/A	N/A	N/A	0
IP pond Water (sampled on 5th June)	N/A	N/A	N/A	6	N/A	N/A	N/A	0

Notes:

Blood samples tested by H7 HI test for detection of H7 antibodies.

H7 RRT-PCR test results given for other sample types (swabs, carcasses and environmental).